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EVALUATION OF CARPET STEAM AND HEAT CLEANERS AS BIOLOGICAL SAMPLING DEVICES

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14. ABSTRACT:
The efficacies of three types of carpet cleaners were evaluated as possible sampling devices for biological contaminants such as *Bacillus anthracis* spores. Residential, commercial, and industrial carpet cleaners were compared for their effectiveness in sampling contaminants. Room-size carpet pieces (8 × 10 ft) were inoculated with aerosolized *B. globigii* spores. Uniform deposition of aerosol spores across carpet surfaces was confirmed using core samples and reference coupon analyses. The industrial unit was the most-effective carpet cleaner (recovering 70–80% of the inoculated spores). Re-aerosolization of spores during sampling was above the infectious dose. The potential use of industrial carpet cleaners as sampling devices is regarded as an important step forward in dealing with wide-area biological consequence management. Additional work is needed to develop this approach into a valuable, readily available sampling capability and to evaluate its applicability for decontamination.

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PREFACE

The work described in this report was started in March 2010 and completed in December 2012.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

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1. INTRODUCTION

In 2001, five envelopes containing a significant amount of anthrax-causing spores of *Bacillus anthracis* were intentionally mailed through the U.S. Postal Service. Over a short time period, this incident resulted in the deaths of five individuals; infection of 17 others; and contamination of 35 postal facilities, commercial mailrooms, and buildings. This attack and its effects demonstrated the consequence of bioterrorism on a limited scale. Congressional inquiries and Government Accountability Office (GAO) investigations in 2005 and 2012 identified two main concerns with the response to this event: (1) reliance on sampling specific locations for the probability of finding spores and (2) the methods used in the sampling and analyses processes. These methods were not validated. The GAO seriously challenged the negative sampling results and decision processes used to establish and substantiate clearance following cleanup after a contamination incident.

Since 2005, many interagency programs have been established to further examine and enhance the capabilities for sampling, decontaminating, and clearing biologically contaminated areas, including the Interagency Biological Restoration Demonstration (Seattle, WA), Wide Area Recovery and Resiliency Program (U.S. Department of Homeland Security [DHS], Washington, DC), Validated Sampling Protocol, and Transatlantic Collaborative Biological Resiliency Demonstration (collaborative effort between the U.S. Department of Defense [DoD], Washington, DC; the U.S. Department of State [DOS] Washington, DC; DHS; and the Republic of Poland). These programs are primarily led by the DHS and DoD but have significant collaboration with a number of other federal agencies, such as the U.S. Environmental Protection Agency (EPA), the U.S. Department of Health and Human Services' (HHS) Assistant Secretary for Preparedness and Response, and the Centers for Disease Control and Prevention (CDC).

The threat of a wide-area release of biological warfare (BW) agents such as *B. anthracis* spores is valid, and the consequences of such a release are perceived to be extremely challenging and daunting in terms of managing individual health and environmental cleanup. Two of the biggest challenges with a wide-area release are (1) recognizing the overlap between response and recovery phases and (2) managing the scale of restoration activities. After a release, one of the first steps in restoration and recovery is to use quantitative sampling to delineate the zone of buildings contaminated with *B. anthracis* spores. Three common methods currently used to collect biological samples and establish the extent of surface contamination are vacuum socks, swabbing, and swiping. A summary of these methods is provided in Table 1.

Of the three methods, only the vacuum sock is suitable for sampling porous and large-area surfaces. Unfortunately, the biological capture efficiencies from porous surfaces using this method were very poor ([10–20%] Ryan, 2011 and Edmonds et al., 2009). Therefore, additional approaches for sampling from porous surfaces are highly desirable. Alternative methods could improve restoration activities in two areas: (1) increased biological capture

efficiencies and (2) availability of additional sampling methods and resources to support rapid recovery and cleanup after a wide-area release.

Table 1. Common Methods for Biological Sampling of Contaminated Surfaces

Method	Surface Type	Surface Characteristics	Validated?	Capture Efficiencies (%)
Swab	Nonporous	Smooth, small nonporous glass and metal, and hard-to-reach smooth surfaces	Yes[a]	15.8–55.0
Wipe	Nonporous	Smooth, large-size nonporous glass and metal surfaces	Yes[b]	24.4–32.4
Vacuum Sock	Porous	Ventilation ducts, carpet, fabric, brick, cinder block, and asphalt	No	10–20[c]

[a] Hodges et al., 2010.
[b] Rose et al., 2011.
[c] Ryan, 2011.

The key rationale for this study was to explore additional methods to sample porous surfaces such as carpets for spores. Applicability of such an approach is expected to expedite the sampling and analysis efforts. The two objectives for this study were

(1) to determine whether wet and dry household or commercial vacuum cleaners provide are adequate for biological sampling porous surfaces; and

(2) to determine which of the three tested cleaners represents the most effective sampling device.

This study evaluated three carpet cleaner models based on their utility as sampling devices and efficiency in extracting *B. anthracis* surrogate, *B. globigii* spores from experimentally contaminated commercial-grade carpets. This study provides quantitative data to support the possible use of such devices for sampling.

2. TEST DESCRIPTION

The following types of steam carpet cleaners were tested:

- industrial grade,
- commercial grade, and
- portable residential unit.

The general approach for this project was to quantify the spores in the following three stages:

(1) Before sampling: Determine the number of spores deposited on the carpet in each run by extracting spores from carpet reference (CR) and glass reference (GC/GR) coupons placed in five different locations before exposure (see Figure 4);

(2) After sampling: Quantify spores in the rinsate in the collection reservoir; and

(3) After sampling: Cut 2 × 5 cm core samples (CS) from the carpet to estimate the residual spore number left in the carpet.

Spores were enumerated in fractional aliquots and normalized to the total volume of spores recovered in the rinsate collection reservoir with the spores extracted from the reference coupons and core-carpet samples. The log colony-forming units (cfus) were computed and used to determine the percent recovery assessments. Aerosol sampling of test environment air during carpet sampling was conducted using glass fiber filters, which were positioned 2, 4, and 6 ft above the carpet surface. Air was sampled at a rate of 15 L/min for 15 min, which resulted in the passage of 225 L of air. The spores collected on these filters were enumerated to estimate the degree of re-aerosolization during the sampling process. In the last few experiments, two additional sets of filters were included. One set of air samples was taken before any air was purged. After overnight settlement of deposited spores, a second set of air samples was taken after air was purged to remove air suspended spores from the chamber. Although accounting for 100% of the deposited spores may be an unrealistic goal, the multiple approach used in this study allowed for a thorough quantitative analysis of the recovery efficiency of each of the three cleaners.

A preparation of fluidized *B. globigii* spores was aerosolized onto a large carpet sample (8 × 10 ft.) and allowed to settle overnight. The target density was 7 logs/ft^2. Reference coupons placed on a carpet were extracted to assess deposition density. Air was purged from the chamber to remove air-suspended spores. A carpet cleaner was used to recover deposited spores from the carpet surface. Four replicate carpet samples were included for each cleaner type.

Some of the differences observed with the various sampling techniques described in literature can be explained by the use of different inoculation methods in testing protocols. Four techniques were commonly used for inoculation of spores onto testing surfaces: aqueous

suspension deposition, aqueous fine-mist deposition using a nebulizer, puff deposition using a metered-dose inhaler (organic propellant as a carrier), and aerosol deposition of milled dry spores. Aerosol deposition of dry milled spores most closely resembles the incident of 2001; therefore, it was the method of choice for the current testing.

2.1 Test Site and Facility

Testing was conducted at the U.S. Army Edgewood Chemical Biological Center (ECBC) by a BioDefense (BD) and Aerosol Sciences Branch (ASB) team. Aerosol deposition and carpet cleaner sample collection were performed in the ASB laboratories. Coupon extraction, sample analysis, and quantitative assessment were performed in the BD laboratories.

The BD biosafety level (BSL) 1 and 2 facilities are fully equipped to handle a broad range of work that requires the safe use and storage of surrogates and pathogens. Since its initial certification in 2001, the facility has functioned as a research and a technology development laboratory in support of DoD chemical and biological programs. The BD branch and its personnel have demonstrated the ability to store and safely handle a variety of BW agents and their surrogates.

Aerosol deposition of spores was conducted in a 64 m^3 BSL-1+ chamber located in the ASB building (Figure 1). Temperature and relative humidity (RH) of the chamber were set using a computer and maintained at 75 ± 5 ºF and 35 ± 5% RH, respectively. Power receptacles inside the chamber were also controlled by this computer. High-efficiency particulate arrestance (HEPA) filters were installed at the inlet to filter air entering the chamber and achieve very low background particle concentrations in the chamber. Similarly, HEPA filters were installed at the exhaust port to filter all particles leaving the chamber.

Figure 1. Aerosol deposition chamber used in this study at ECBC.

2.2 Test Technologies: Carpet Steam and Heat Cleaners

Numerous varieties of carpet steam cleaners are available for use in residences and commercial facilities. Portable residential units may be purchased at hardware or home stores. Commercial and industrial units are typically stocked by specialty companies. The key differences between residential-, commercial-, and industrial-grade units are the temperature and pressure levels, which tend to be higher for industrial-grade units. All the units that were tested have the ability to heat the aqueous solution being used; however, none of these units produce steam horsepower.

The residential carpet cleaner used for this testing was the Bissell (Grand Rapids, MI) ProHeat 2× CleanShot 9500. Unlike many residential units, this model includes a built-in heater that increases the temperature of hot tap water by as much as 25 °F. The unit has a 1 gal tank for rinsate collection.

The commercial-grade steam cleaner used for this testing was the Xtreme Power XPH-9650 carpet extractor manufactured by Daimer Industries, Inc. (Woburn, MA). It can be operated at up to 500 psi, and the cleaning solution can be heated up to 210 °F. It has a 15 gal recovery tank in which the rinsate is collected.

The industrial steam cleaner used for this testing was the TM Sapphire 370ss unit (Sapphire Scientific, Prescott, AZ). This is a truck-mounted carpet cleaning machine with a 20 horsepower gasoline engine. The unit included a vacuum blower, solution pump, heat exchangers, and a 90 gal waste tank. The unit allowed complete temperature control up to 250 °F.

The units are shown in Figure 2, and related data are listed in Table 2.

A

B

C

Figure 2. Three capet cleaners: (A) residential unit (Bissell), (B) commercial unit (Daimer), and (C) industrial unit (Sapphire).

Table 2. Summary of Vacuum Cleaners Tested in This Study

Cleaner Type	Temperature (°F)	Pressure (psi)	Waste Tank (L)	Vacuum Flow (CFM)[a]	Cost ($)	Comments
Residential (Bissell ProHeat CleanShot 9500)	25[b]	NA	3.7	NA	300	NA
Commercial (Xtreme Power XPH-9650) by Daimer Industry	210	500	56	200	5,000	NA
Industrial (TM Sapphire 370ss)	250	1500	340	317	30,000[c]	Can be trailer or truck mounted

NA: not applicable.
[a] CFM: cubic feet per minute.
[b] Above the ambient.
[c] The cleaning unit cost $25,000. The additional $5,000 was for a trailer.

2.3 Carpet Specifications

Shaw carpeting, Viking style, Stingray 12 ft width carpet (purchased from Home Depot, Aberdeen, MD) was used. This carpeting was representative of typical flooring found in office spaces and was 100% level loop olefin with a polypropylene backing. The carpet sections used for each run were 8 × 10 ft. The sections were placed on plywood board to allow coring of small carpet pieces after vacuuming. For each run, a new carpet section was used; four replicate carpet sections were used with each technology.

3. SPORE INOCULUM

The fluidized milled *B. globigii* spore preparation used for this project was procured from Dugway Proving Grounds. A specifications sheet with certificate of analysis, which documented quality assurance/quality control of the spore preparation, was presented upon delivery. Spores were prepared by milling the preparation until the particles consisted of single spores and then adding fluidizer. Before testing, a known mass of dry powder (20 mg) was suspended in 10 mL volume of 0.01% Tween-80. The spores were serial diluted and plated on tryptic soy agar (TSA) plates for enumeration. Three separate enumerations were performed to determine the number of viable spores per gram of dry powder. Appropriate dilutions were plated on duplicate TSA plates. Colony-forming units were counted and averaged from two plates. The titer (colony-forming units per milliliter) was determined by using eq 1:

$$\text{Total cfu/mL} = N\ (\text{average cfu}) \times \text{dilution factor} \times \text{volume factor} \quad (1)$$

3.1 Aerosol Deposition of Spores

The fluidized spores ~100 mg (by weight) were aerosolized onto the carpet using two fluid pneumatic sonic nozzles (SRI International, Menlo Park, CA [Figure 3]). One nozzle was connected to the compressed air that was released through a small annular opening. The other nozzle was connected to the powder to be aerosolized. The low pressure created in the exit region as a result of the airflow caused powder to be pulled through an axial tube at a very low feed rate because of the Bernoulli Effect (Beiser, 1991). The desired air-to-powder mass ratio was 80–100:1.

For each test, an 8 × 10 ft carpet section was contaminated with $1 \times 10^7/ft^2$ spores or 8×10^8 spores. To deliver the intended concentration, each run required a deposition from approximately 1.6×10^9 to 2.0×10^9 spores in the chamber (based on the entire chamber surface area). It was expected that some spores would remain in the nozzle and some would be unaccounted for because of the surrounding surfaces (e.g., extra chamber floor space that was not covered by the carpet section and the walls and ceiling). No attempt was made to account for these spores.

Aerosol was generated inside the chamber. The chamber air was mixed using fans after or during aerosol generation to achieve a uniform aerosol concentration. Previous tests showed that mixing the aerosol in the chamber for 1 min was adequate to achieve a uniform aerosol distribution. After the aerosol deposition process, the fans were turned off and the spores were allowed to settle overnight. Before sample collection the next day, a bleach solution was used to decontaminate the spores on the chamber floor surface that was not covered by carpet.

The aerosol chamber was cleaned between the runs by exhausting the chamber air through the HEPA filters and by pumping HEPA-filtered air into the chamber. The maximum amount of airflow exhausted from the chamber by the exhaust pump was approximately 2×10^4 L/min. There was also a small recirculation system that removed air from the chamber, passed it through a HEPA filter, and delivered it back to the chamber. This system was useful when the aerosol concentration in the chamber needed to be reduced incrementally. In addition, the chamber walls and floor were wiped down with 10% bleach between runs. If the air sampled between runs showed a significant number of spores, the chamber was decontaminated with vaporous hydrogen peroxide.

Between each run, the carpet cleaners were cleaned by thoroughly rinsing the storage reservoir and all the components three times with sterile 0.01% Tween-80 solution. After the three sets of rinsing, the reservoir was filled with sterile solution and tested for viable spores. The number of viable spores was >10 spores/mL. Even though the cleaners were not free from spores, the carryover residue was estimated to be insignificant (several hundred), considering the inoculation density of ~7 logs/ft^2. Bleach treatment was not considered to be essential because of the effectiveness of the repetitive rinsing steps, which showed low numbers of residual spores and the negative impact of residual traces of bleach on viable spores in each successive run. However, if an highly contaminated area had been sampled before a lower contaminated area, it would have been necessary to rinse the reservoirs with bleach (6000 ppm of free available

chlorine [FAC]) and then rinse three more times with 0.01% Tween 80, until only bleach residue and spores could be detected in the reservoir sample.

3.2 Carpet Sampling and Spore Extraction Process

One cleaner type was used for each test run, and each technology was tested in quadruplicate. Therefore, testing required 12 runs. The filtered sterilized surfactant (Tween-80) in sterile deionized water was added to the carpet cleaner reservoir of the technology tested at a final concentration of 0.01% before the start of a run.

For the spore extraction procedure, the carpet cleaner was operated over the entire surface of the carpet section twice. The cleaners were used in accordance with manufacturer's recommendations. The surfactant was first sprayed onto the carpet surface, and then, the wand or cleaning unit was used in a left-to-right pattern to sample the spores from the full length of the carpet. This procedure was repeated at a 90º angle to the first pass. Care was taken to ensure that the steam cleaner operator or other personnel did not step onto the carpet during the test runs. This approach helped to prevent unwanted cross-contamination during the process.

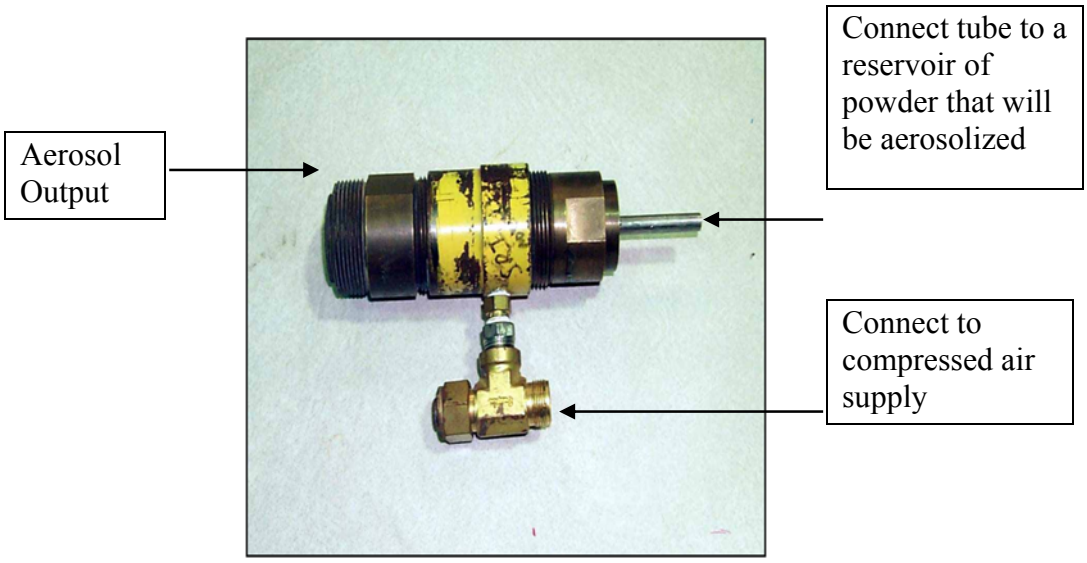

Figure 3. Sonic nozzle used for aerosol generation.

3.3 Rinsate Sample Analysis

Maximum rinsate volumes collected with the three types of cleaners were 4–5 L (industrial), 1–2 L (commercial), and 0.5–1 L (household). However, the maximum collection capacity for these devices was much higher (i.e., 300 L [industrial], 30 L [commercial], and 3.7 L [household]). The baseline studies established an adequate volume of solution to use for making two passes over the carpet. Three subsets of the collected rinsate were analyzed by serially diluting it with 0.01% Tween-80 solution in the ratio of 1:10 up to 10^{-3}.

Then 100 µL aliquots were directly plated onto two TSA plates. The TSA plates were incubated at 37 ºC for 18 ± 4 h. Dilutions were made in. The colony-forming units were counted using the Q count (Advanced Instrument, Inc., Norwood, MA) instrument.

4. REFERENCE COUPONS

Each test run included five reference coupons each of carpet and glass. These coupons were used to determine the quantity of spores deposited during each run. Each coupon was 1 × 2 in. in size. CR coupons were placed in each 4 ft^2 section (Figure 4). Five glass coupons were positioned with the CR coupons. Coupons were placed on the carpet section before the aerosol-deposition procedure. After the overnight settling period, the coupons were removed and placed in 20 mL extraction buffer (0.01% Tween-80). The reference coupons were extracted using a 10 min sonication and 2 min vortexing procedure. The samples were plated on duplicate plates after appropriate dilution as previously described (Rastogi et al., 2009 and 2010).

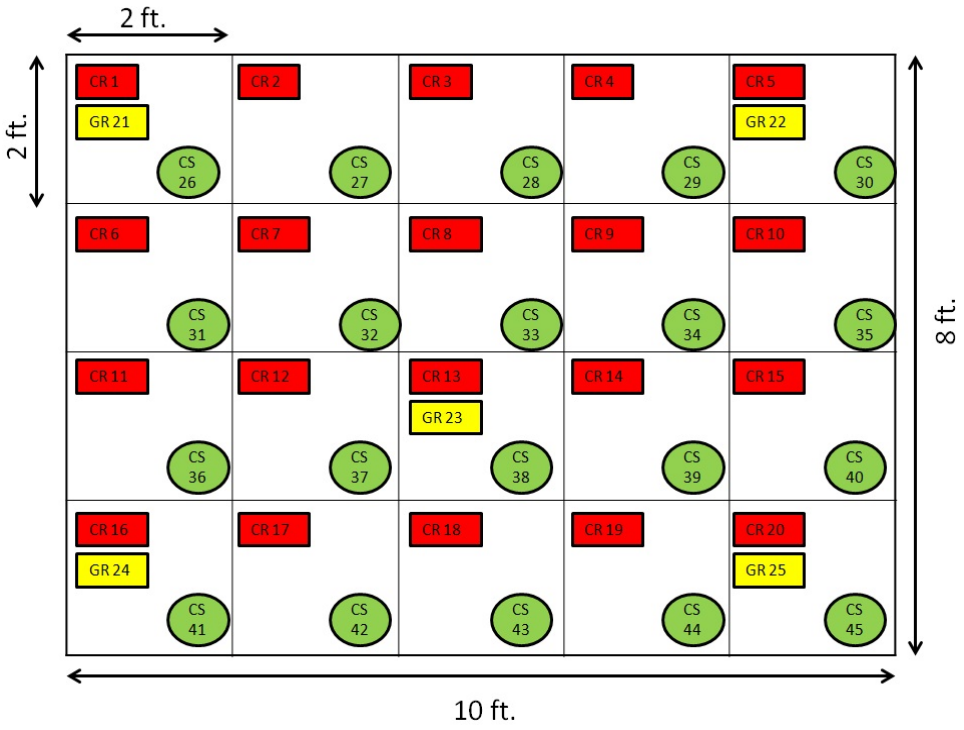

Figure 4. Sampling grid (CR, GR, and CS).

4.1 CSs

To estimate the residual number of spores that were not recovered during sampling, CSs were taken from the carpet section used for each test. A total of five CSs were taken from each carpet tested. A sharp blade was used to cut around a steel template (2 × 5 cm in size). The core carpet samples were placed in a 50 mL conical tube containing 20 mL 0.01%

Tween-80. The samples were processed using the same procedure that was used for the reference coupons.

4.2 Air Sampling

Because spores were expected to re-aerosolize during the sampling process, glass fiber filters for air sampling were operated (1 L/min for 15 min) at 2, 4, and 6 ft above the carpet surface. After collection, the filters were extracted and analyzed for viable spores. Glass fiber filters (47 mm) were placed in the proper positions after the overnight spore-settling period but before the carpet-extraction process. After the carpet-extraction process was completed, the filters were placed in a 50 mL tube containing 20 mL 0.01% Tween-80 and processed in the same manner as the reference coupons and CSs. The spore numbers were quantified by dilution plating. This analysis was semi-quantitative because the recovered spores were not acclimatized to the air passed through the glass fiber filters. The data were generally used to evaluate spore aerosolization during the sampling process.

The original plan was changed to only five reference coupons and five carpet CSs after the baseline.

4.3 Samples Collected during Each Test Run

The test matrix for each run included: five CR, five GR, and three rinsate analysis samples. In addition, five carpet CSs were also analyzed. The test matrix is presented in Table 3.

Table 3. Sample Designations Detailed in the Testing Matrix

CR Coupons	GR Coupons	Carpet CS	Rinsate Samples[a]	Pre-Purge[b]	Pre-Sample[c]	Air Samples[d]
CR 1	GR 11	CS 21	RS 31	PP 41	PS 41	AS 41
CR 2	GR 12	CS 22	RS 32	PP 42	PS 42	AS 42
CR 3	GR 13	CS 23	RS 32	PP 43	PS 43	AS 43
CR 4	GR 14	CS 24	NA	NA	NA	NA
CR 5	GR 15	CS 25	NA	NA	NA	NA

[a] RNs: rinsate sample code.
[b] PP: pre-purge sample code.
[c] PS: pre-sample code.
[d] AS: air sample code.
NA: not applicable.

4.4 Data Reduction

Primary data used for quantitative estimations were collected from two sources, the reference coupons and the cleaner rinsate. In addition, air samples on glass filters were collected for semi-quantitative estimation of re-aerosolization. All reference coupons (5 glass and five carpet) were extracted in 20 mL of extraction buffer (0.01% Tween-80).

Data reduction included calculating the total viable spores that were recovered.

Each reference coupon was 10 cm^2. The cfus recovered from each reference coupon were calculated using eq 2:

$$\text{Average cfu (2 plates)} \times \text{dil. factor} \times 10\ (100\ \mu\text{L was plated}) \times \text{extract vol. (20 mL)} \quad (2)$$

The following average density and standard deviation from five CR coupons was calculated:

Area per coupon	=	10 cm^2 (or 1.55 $in.^2$)
Area per carpet	=	80 ft^2 (11520 $in.^2$ or 74322 cm^2)
Factor (extrapolation)	=	7432 (carpet area cm^2/coupon area cm^2)
Total cfu/carpet area	=	7432 × average cfu/5 coupons

A 100 µL aliquot from three batches of collected rinsate was diluted and plated on TSA plates. Total colony-forming units for the rinsate samples were calculated using eq 3:

$$\text{Average cfu/mL} \times \text{total rinsate volume (mL)} \quad (3)$$

The total number of spores estimated in collected rinsate was used to calculate the sampling efficacy.

The measurements for air sampling are not quantitative. The intent for calculating these measurements was to confirm the re-aerosolization prediction during the sampling process and to confirm the height at which these spores aerosolize.

The sampling efficacy of each of the three technologies was calculated using eq 4:

$$\text{Total cfus collected in the rinsate/total cfu per carpet} \times 100 \quad (4)$$

Averages and statistical variability were calculated by using data from four replicate runs. The data were used for determining the effectiveness of each tested technology in reducing contamination levels.

4.5 Data Quality Objectives (DQOs)

DQOs define the critical measurements that are performed to address stated objectives and to establish accepted limits for potential errors associated with the proposed sampling study. Measuring the colony-forming unit and rinsate volume was considered necessary for accomplishing the project objectives.

The data quality indicators (DQIs) listed in Table 4 were critical for quantifying how well the collected data met the DQOs. Table 5 presents the acceptance criteria for the critical measurements.

Table 4. DQIs for Critical Measurements

Measured Parameter	Analysis Method	Accuracy	Detection	Completeness Goals (%)
Spore counts (cfu)	Counting using QCount (image recorded)	±5%	1	100
Rinsate volume	Visual observation of graduates on a carboy (volumetric)	±10%	1 mL	

Table 5. Acceptance Criteria for Critical Measurements

Measured Parameter	Target Value	Precision RSD[a] (%)
Reference coupons	1–300 cfus per plate	50 (replicate plates)
Rinsate volume	Varies with technology type	20

[a] RSD: relative standard deviation.

4.6 Safety and Environmental Protection Requirements for Test

All personnel performing the work were familiar with the standard laboratory techniques and equipment operation required for use during this project. Additional specialized training and certifications for the use of the equipment and instruments were identified and provided by each vendor on site.

4.7 Quality Control Requirements for Test

The tests were conducted in accordance with standard good laboratory practices. The BD Branch has a quality assurance system in place and a number of internal operating procedures were strictly followed to complete this project.

5. RESULTS

5.1 Baseline Studies

One basic parameter in this study was to ensure a uniform spore deposition across the carpet area (80 ft^2). In the preliminary testing, powder was released through the sonic nozzle, uniform mixing was ensured by using fans, and spore settling-time parameters were controlled. As seen in Figure 5, analyses of 20 reference coupons (CR, GC/GR, and CS cut from carpet) spread over the carpet area showed that spore deposition was spatially uniform. In later experiments, only five CR or GR coupons were placed ahead of spore deposition. The data obtained in the baseline experiments established that spore deposition density was around 7.5 ± 0.25 log (cfu)/ft^2. Figure 5 graphically summarizes the analyses of the two types of reference coupons: 20 CRs and 5 GC/GR coupons. The results clearly showed that the spores were uniformly deposited across the carpet area. Based on this result, only 5 CR coupons were used in the 12 experimental runs.

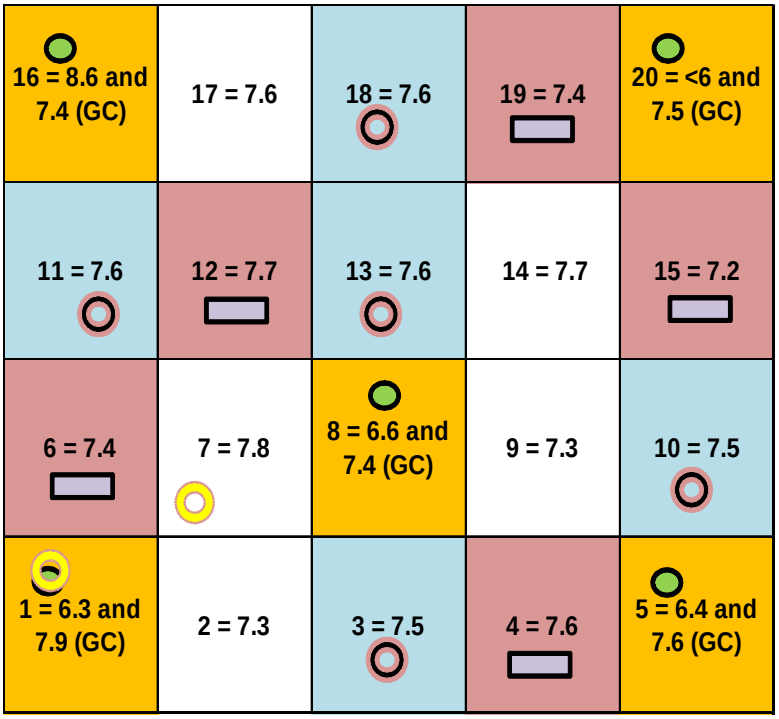

Figure 5. Graphic representation of 20 CR coupons (numbered in each rectangle) and 5 GC/GR coupons.

Each carpet (8 × 10 ft.) was divided into 2 × 2 ft sections, and each section was given a number between 1 and 20. Five reference carpet coupons were laid in sections 1, 5, 8, 16, and 20. Five GR coupons were positioned with the CR coupons. Five carpet pieces were cored out from sections 2, 7, 9, 14, and 17. The log for cfu/ft^2 is indicated in each section.

5.2 Spore Deposition Density

The spore-loading density data from a total of 12 runs in these experiments were pooled, averaged, and analyzed as CR, GR and CS coupons cut out after sampling. The results are summarized in Figure 6, and as shown in the graph, there was a good correlation (~9 logs/carpet area) between the two reference coupon types (i.e., glass and carpet). Each bar represents the average of 60 samples with <0.2 log standard deviation. Therefore, the spore-deposition technology is well reproducible.

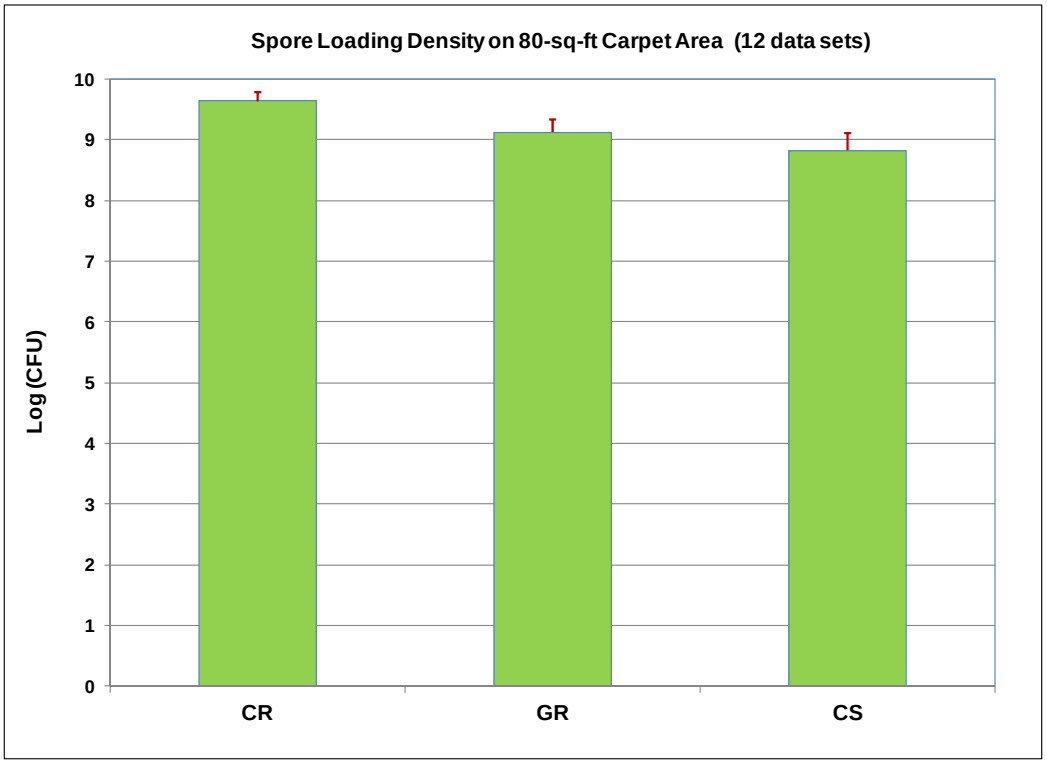

Figure 6. Graphic summary of spore-loading density over carpet area based on a dataset of 60 samples.

5.3 Recovery Efficiency

The three cleaner units had different capacities to hold and collect the wash or rinsate solutions. Figure 7 summarizes the volume of rinsate collected by each of the three cleaner types. The residential unit collected ~0.5 L, the commercial unit collected ~1.5 L, and the industrial unit collected ~8.5 L of rinsate volume. The collection efficiencies depended upon spray volumes and, more importantly, the suction pressures achieved by the three unit types. Based on the initial amount of solution used, the collection efficiencies were estimated to be ~30–40% for the residential unit, 40–50% for the commercial unit, and 70–80% for the industrial unit.

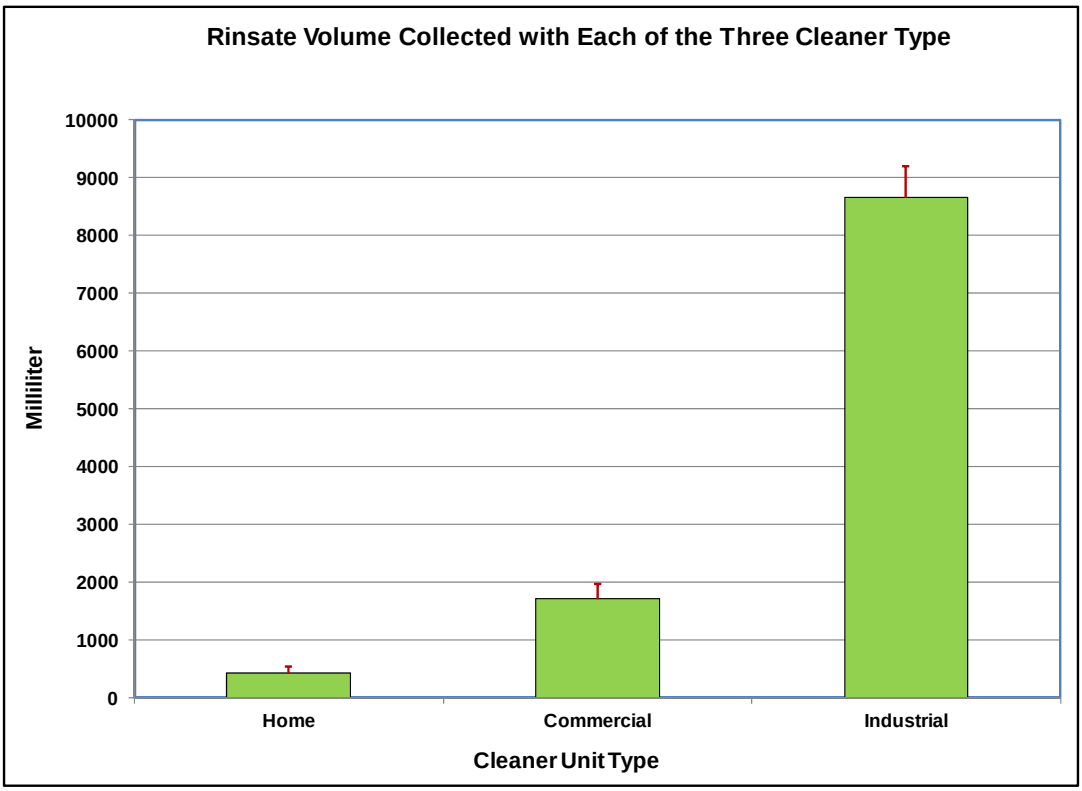

Figure 7. Average rinsate volume collected, based on a dataset of four samples.

Spores recovered from reference coupons and core carpet samples using the Bissell residential unit from four runs are graphically summarized in Figure 8. A strong correlation between the two reference coupon types is evident. Typically, recoveries from the core carpet samples were about 0.5–1.0 log less than those from the GR coupons. Overall, the residential unit does retrieve spores, but the efficiency is ~35%. As a sampling device, the residential unit is comparable to currently used sampling methodologies.

Figure 9 summarizes the spore recovery averages from a set of four runs using the commercial unit. These results were similar to those with the residential unit. Even though the suction pressure and rinsate volume collected by the Daimer unit were superior to the residential unit, the spore recovery was only marginally better.

Figure 10 summarizes the spore recovery from a set of four runs using the industrial Sapphire unit. A correlation between the spores deposited on the two reference coupon types was observed. However, relative to the other two cleaner types, the rinsate appeared to collect more spores, which resulted in less spores recovered from the core samples (≥1.5 logs). A significant number of spores were still left on the carpet, much like other sampling devices. These findings are consistent with other similar studies performed on carpets (Ryan, 2012).

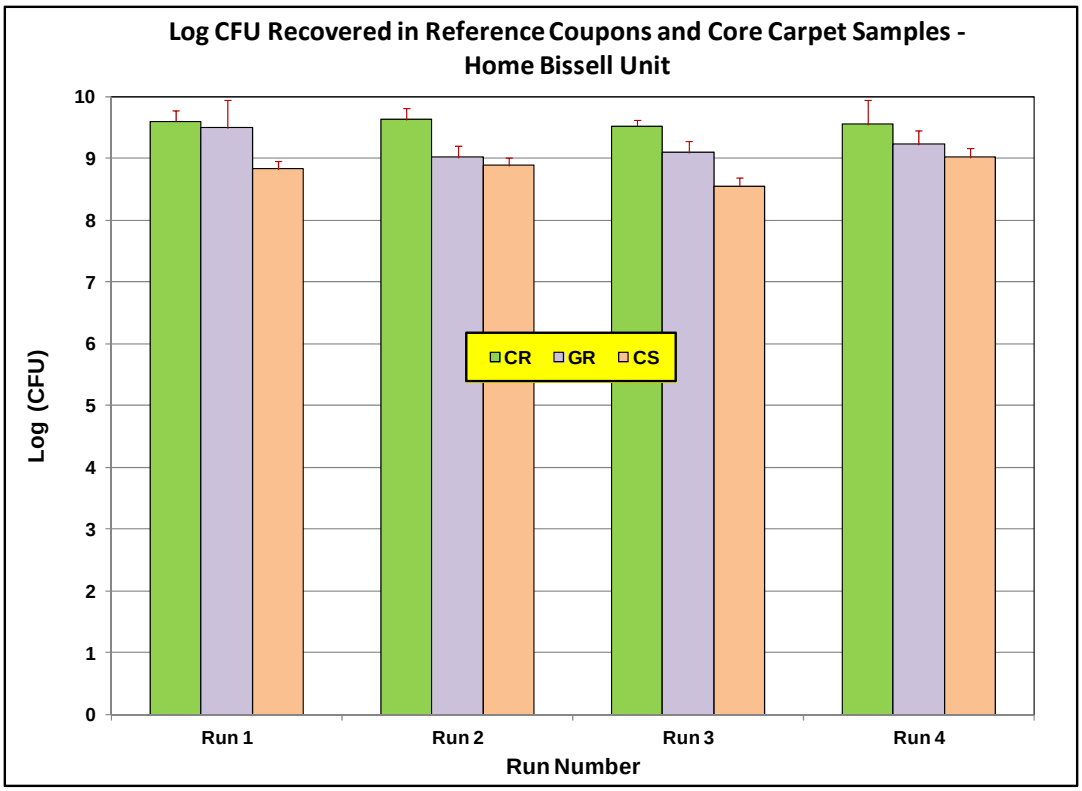

Figure 8. Spore recovery using a residential unit, based on a dataset of four samples.

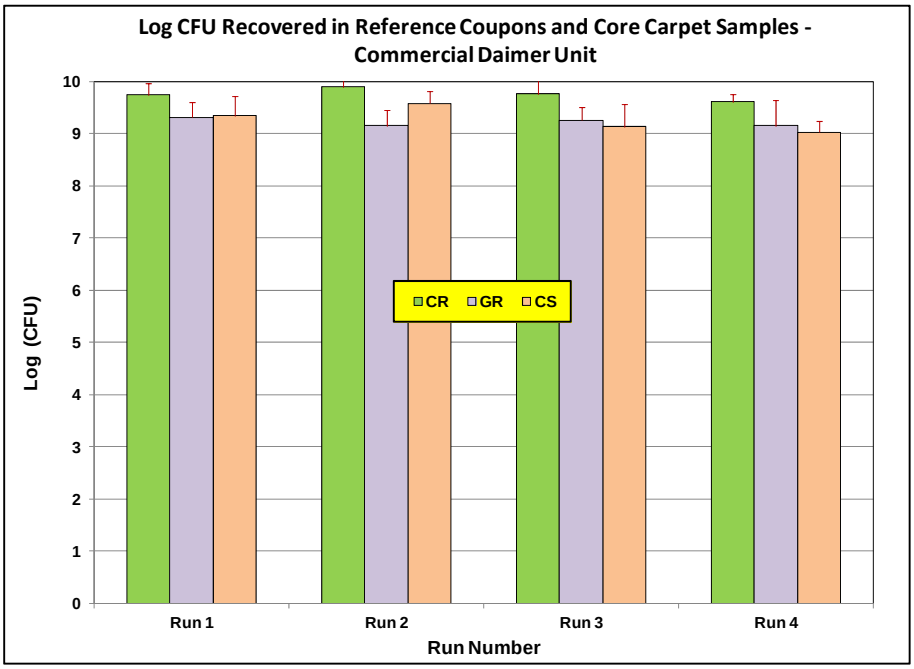

Figure 9. Spore recovery using a commercial unit, based on a dataset of four samples.

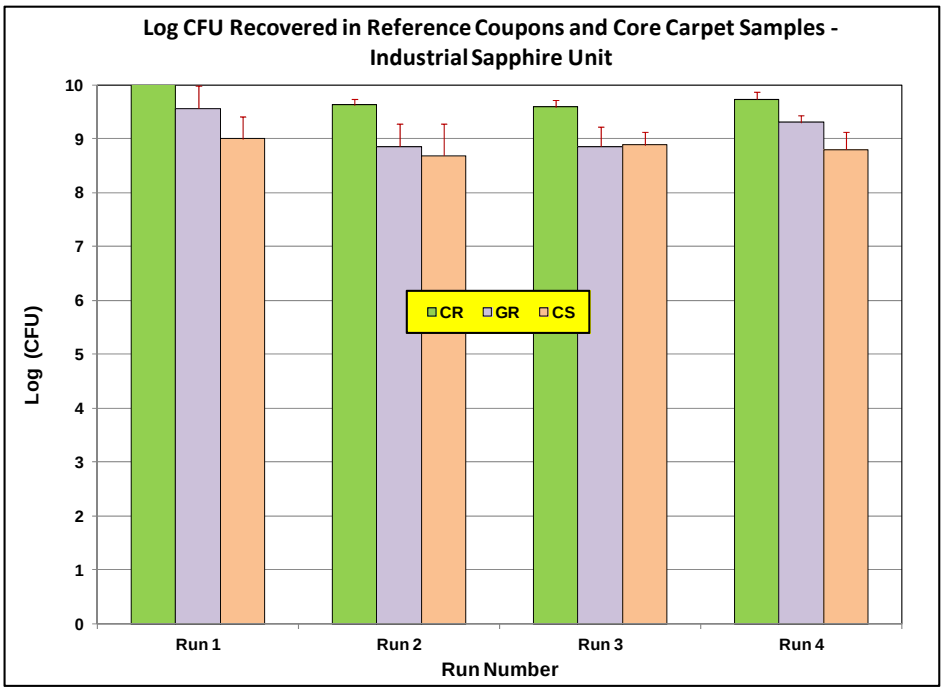

Figure 10. Spore recovery using an industrial unit, based on a dataset of four samples.

The spore recovery efficiencies, relative to the number of spores deposited, was estimated for each of the three cleaner types, and the results are summarized in Figure 11. As shown, the residential and industrial units are roughly 30–35% efficient in spore recovery, and the industrial unit is >70% efficient in spore recovery. This degree of spore sampling efficiency is higher than previous estimates for any other sampling device.

In addition, tests were performed to determine whether spores can aerosolize during the cleaner sampling process; however, this assessment was not fully quantitative. Glass filter fibers were placed at three heights. Air was sampled through the filter for 15 min at 15 L/min. The spores collected from the filters were quantified. Figure 12 summarizes the spores recovered at the pre-purge, pre-sample, and air sampling stages during the cleaner sampling process. The results showed that 4–5 spore logs were suspended in the air during carpet cleaning. Typically, human air intake during breathing is 15 L/min. The number of spores inhaled in 15 min is therefore expected to be well above the infectious dose of 8,000–50,000 (Public Health Agency of Canada, 2011; Ottawa, Ontario, Canada).

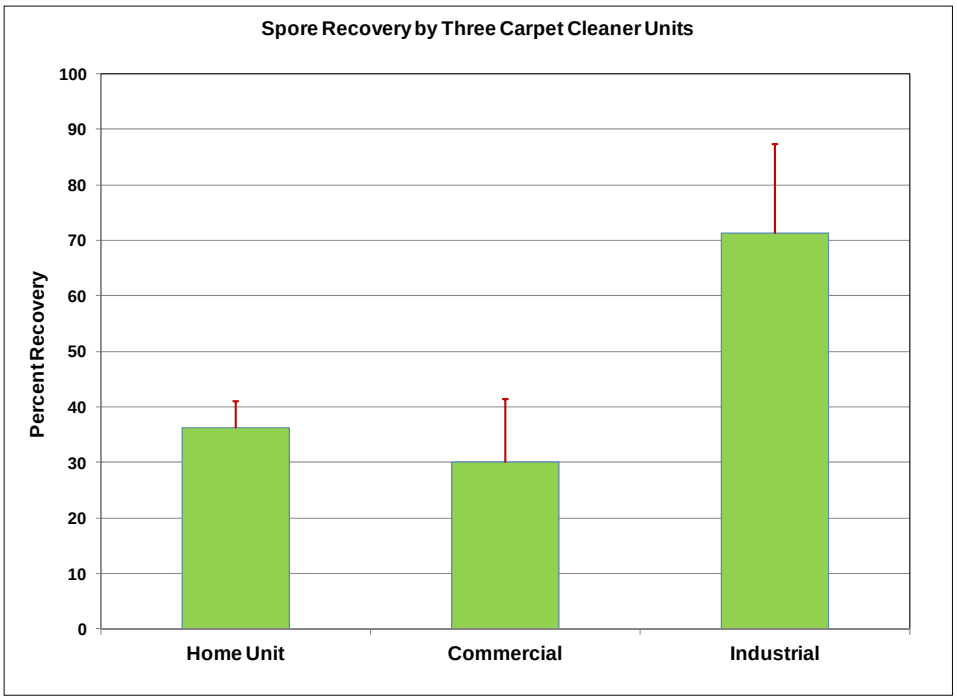

Figure 11. Spore recovery efficiencies by the three cleaner types.

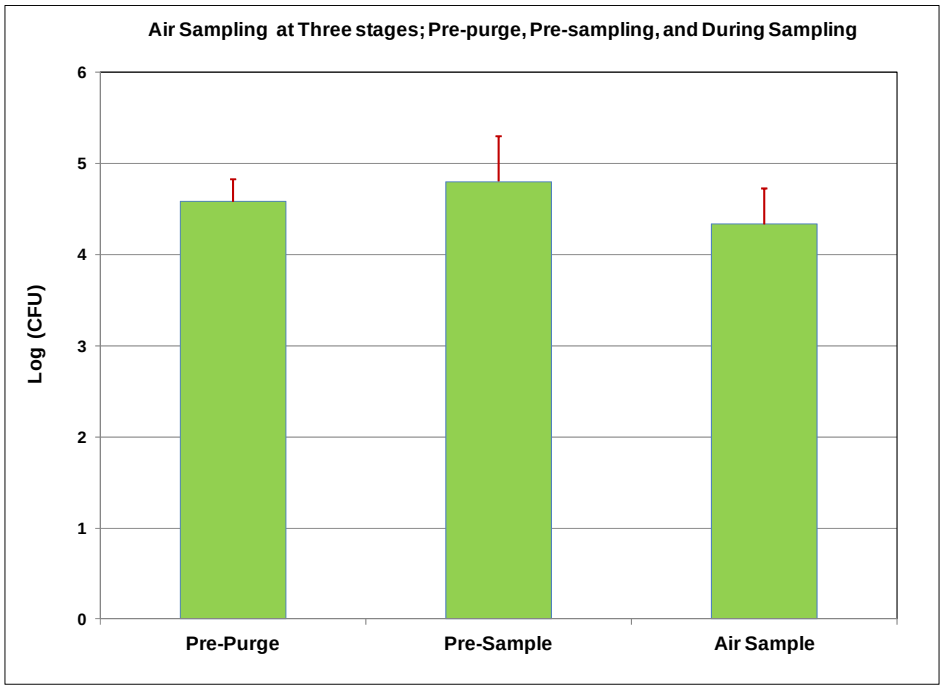

Figure 12. Spores aerosolized at different stages during a run.

6. CONCLUSIONS AND DISCUSSION

Two clear objectives of the present study were to explore efficacy of sampling devices (i.e., three types of carpet cleaners for the collection of spores from porous surfaces). The study was conducted indoors within a chamber, under well-controlled ambient conditions. It was observed that the inoculation density in all 12 runs across the entire 80 ft^2 carpet area was comparable (7–7.5 logs/ft^2).

While the residential and commercial units were only marginally effective (30–40% efficiency), the industrial unit was highly effective in reducing contamination levels by 70–80%. Therefore, in the event of large-area contamination, the industrial unit should be used as a primary high-throughput sampling device. Because these are commercially available units that have dual-use purpose, any additional investment would be limited to training personnel for sampling hazardous material. Although the dataset collected in this study is encouraging, additional work is needed to validate these results and to support such readily available cleaners as sampling devices. Future testing should focus on the following research:

- sampling with different loading densities (i.e., 4 and 6 logs/ft^2 challenge levels);
- a multi-laboratory study involving federal (e.g., EPA) or commercial laboratories working concurrently to validate results;
- using bleach (6000 ppm FAC, pH 7) instead of Tween-80 solution in the reservoir to test applicability of carpet cleaners in decontamination and source reduction; and
- similar studies using other porous and nonporous surfaces to confirm collection efficiencies on a broader scale.

In this study, testing was performed using only dry spore powder, which represents one of the most probable forms of release by sophisticated terrorists in a biological attack. The data collected here are therefore pertinent to one type of biological incident. Additional sampling data need to be collected using other forms of spore releases, such as aqueous and air mist of spores or spore puffs.

LITERATURE CITED

Beiser, A. *Physics*, 5th ed.; Addison Wesley Longman, Inc.: Reading, MA, 1991.

Edmonds, J.M.; Collett, P.J.; Valdes, E.R.; Skowronski, E.W.; Pellar, G.J.; Emanuel, P.A. Surface Sampling of Spores in Dry-Deposition Aerosols. *Appl. Environ. Microbiol.* **2009**, *75* (1), 39–44.

Government Accountability Office. *Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results*; GAO-05-251; Government Accountability Office: Washington, DC, 2005.

Government Accountability Office. *Anthrax Detection: DHS Faces Challenges in Validating Methods for Sample Collection and Analysis*; GAO-12-488; Government Accountability Office: Washington, DC, 2012.

Hodges, L.R.; Rose, L.J.; O'Connell, H.; Arduino, M.J. National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces. *Jour. Microbiol. Methods* **2010**, *81*, 141–146.

Public Health Agency of Canada. *Bacillus anthacis*; MSDS 12; Public Health Agency of Canada: Ottawa, Ontario; http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds12e-eng.php (accessed 1 December 2012)

Rastogi, V.K.; Wallace, L.; Smith, L.S.; Ryan, S.P.; Martin, G.B. Quantitative Method to Determine Sporicidal Decontamination of Building Surfaces by Gaseous Fumigants, and Issues Related to Laboratory-Scale Studies. *Appl. Environ. Microbiol.* **2009**, *75*, 3688–3694.

Rastogi, V.K.; Ryan, S.P.; Wallace, L.; Smith, L.S.; Shah, S.S. Martin, G.B. Systematic Evaluation of Chlorine Dioxide Efficacy in Decontamination of Building Interior Surfaces Contaminated with Anthrax Spores. *Appl. Environ. Microbiol.* **2010**, *76*, 3343–3351.

Rose, L.J.; Hodges, L.; O'Connell, H.O.; Noble-Wang, J. National Validation Study of a Cellulose Sponge Wipe-Processing Method for Use after Sampling *Bacillus anthracis* Spores from Surfaces. *Appl. Environ. Microbiol.* **2011**, *77*, 8355–8359.

Ryan, S. U.S. Environmental Protection Agency's National Homeland Security Research Center, Research Triangle Park, NC. Private communication, 2011.

U.S. Environmental Protection Agency. *Assessment of Liquid and Physical Decontamination Methods for Environmental Surfaces Contaminated with Bacterial Spores: Development and Evaluation of the Decontamination Procedural Steps*; EPA/600/R-12/025; U.S. Environmental Protection Agency: Washington, DC, 2012; http://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=505995 (accessed 1 December 2012).

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ACRONYMS AND ABBREVIATIONS

AS	air sample code
ASB	Aerosol Sciences Branch
BD	BioDefense
BSL	biosafety level
BW	biological warfare
cfm	cubic feet per minute
cfu	colony-forming unit
CR	carpet reference
CS	core sample
DHS	U.S. Department of Homeland Security
DoD	U.S. Department of Defense
DQI	data quality indicator
DQO	data quality objective
ECBC	U.S. Army Edgewood Chemical Biological Center
EPA	U.S. Environmental Protection Agency
FAC	free available chlorine
GAO	Government Accountability Office
GC/GR	glass reference
HEPA	high-efficiency particulate arrestance
PP	pre-purge sample code
PS	pre-sample code
RH	relative humidity
RN	rinsate sample code
RSD	relative standard deviation
TSA	tryptic soy agar

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